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*Full Length Research Paper*

# Chromosome studies in Cashew (*Anacardium occidentale* L.)

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**Despite the increased cultivation of cashew as a commodity crop in sub-Sahara Africa, Asia and South America there are few chromosome studies on it. The present study investigates number, structure and behavior of chromosome in cashew populations growing in Nigeria. Cytological examination of these populations revealed a diploid and haploid chromosomes of 2n = 42 and n = 21 respectively. The karyotypes were mostly symmetric, composed mainly of metacentric pairs and several sub-metacentrics. Similarity in the morphology, number and behavior of the chromosomes in the accessions from different populations or origin attests to the degree of genetic closeness of the selections. This probably indicates high potential for use as parents in the breeding and improvement of cashew with very limited cross-incompatibility barriers (free gene exchange). Polymorphism in chromosome number was not recorded among these cashew selections.**

**Keywords:** *Anacardium*, cashew, chromosome, karyotype.

## INTRODUCTION

Cashew is a dicotyledonous evergreen tree with a morphologically polymorphous species that has been reported with polymorphic chromosome number 2n = 24, 30, 40, 42 (Deckers et al., 2001). The genus *Anacardium* is a native to latin America and has a primary centre of diversity in Amazonia and secondary one in the Planalto of Brazil. Behrens (1998) described the crop as a tropical tree species that originated from South America. Natural occurrences of cashew have been reported for Mexico to Peru and in the West Indies. The crop was introduced into India, the East Indies and Africa by the Portuguese explorer in the 16th century. Thereafter, exploitation of cashew for its fruits (nut and apple) among local people appears to have been the pattern for more than 400 years in Asia and Africa (Mitchell and Mori, 1987).

Cashew is a small-medium-sized tree of savannah habitat that can grow from 6 - 15 m tall with canopy diameter ranging from 10 - 15 m (Ohler, 1979; Masawe et al., 1998; Aliyu, 2004). It is cultivated for its kernels, shell liquid and apples. The tree has a short-long greyish-brown trunk with spreading branches and a tap-root system, which penetrate very deep into the soil profile and lateral roots that sometimes extends to a radius twice that of the canopy. Cashew is an out-crossing species with pollination being carried out by insects. Although, some level of self-pollination has been recorded but it appears that the degree vary with genotype and environment (Westergaard and Kayumbo, 1970; Ohler, 1979; Aliyu, 2004). The yield of mature cashew trees may varies from less than 5 kg/tree/year to about 40 kg/tree/year due to the genetic variability of the tree populations (Martins and Kasuga, 1998; Aliyu, 2004). Cashew exhibits genetic variability in various character-ristics such as size and form of tree, colour of apple (yellow, orange, or red), disease resistance and fruit-bearing capacity.

Introduction of cashew in Nigeria dated back to the 16th century (Woodroof, 1967; Ohler, 1979), but commercial planting started in the mid-1950s with establishment of large scale plantations with Indian introductions by the then Western Nigeria Development Corporation (WNDC) (Akinwale and Esan, 1989). However, with mandate given to Cocoa Research Institute of Nigeria (CRIN), Ibadan in 1971, to carry out genetic improvement into cashew, germplasm materials were collected from this plantation and selection for improvement was carried out for

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the identification of high yielding genotypes. Despite continued efforts by the Institute to improve the crop, cashew production in Nigeria is still being confronted with problem ranging from irregular tree yield and low quality nuts. In pursuant of the quality improvement in the crop species, the genetic base of the CRIN collections was broadening in the 1980s' with the introductions of Brazilian populations characterized by large and bold nuts.

As part of efforts to effectively utilize the enhanced cashew genetic resource growing in the country, morphogenetic diversity study was carried out on two major populations (Brazilian and Indian) between 2000 and 2002. The study revealed that Indian accessions are potentially prolific and consistence in fruiting, but with characteristic small-medium sized nuts and low-premium kernels. Meanwhile, Brazilian accessions produce large nuts with potentially good quality kernels, but very low and inconsistence in fruit yield (Aliyu, 2004). Based on the report of this diversity work, a recurrent selection breeding strategy has been developed that will involve the use of hybridization of identifiable promising genotypes as parents. The wide variability in germplasm collections of cashew offers opportunities for the exploitation of useful genes for improvement of the crop. However, to achieve the desired improvement through this breeding strategy, knowledge of basic cytology (number, structure and behaviour of chromosomes and pollen grain fertility) of these selections is very essential.

However, cytological and breeding investigations in cashew (*Anacardium occidental* L.) are few compared with other crops. Hutchinson and Dalziel (1954) reported diploid chromosome number of 2n = 42 in the crop species. By comparison with other tropical industrial crops as oil palm, coffee, cacao and tea, very little cashew-improvement research has been done, owing to lack of adequate knowledge of cytology and genetics of the crop. The importance of cytological information to crop improvement cannot be overemphasized. Cytological studies have help a lot in resolving the origin and evolution of plant species. Since the basis of improvement is based on variation, and variation has both genetic and non-genetic components, comparative work should provide useful data for solving the problems of low fertility, incompatibility etc, which are key components in tree crop breeding. These two variations bring about changes in the chromosome either structurally or morphologically are bound to affect the DNA components and therefore have genetic consequence. It has also been known that chromosomal or cytological studies help in determining the path of evolution of new species. Cytogenetics has been employed in agriculture for the development of improved cultivars especially in identifying the cause(s) of infertility in organism.

This work reports number, structure and behaviour of chromosome of two cashew populations growing in Nigeria. The significance of the results is discussed in relation to hybridization and improvement work in cashew.

## MATERIALS AND METHODS

Mature nuts of cashew used for this study are ten representatives each of Indian and Brazilian populations, collected from their primary centres of introduction in Nigeria. The Indian cashew population was first planted in large scale by Western Nigerian Development Corporation between 1954 and 1956 at Iwo, Osun State (Lat. 07° 15$^1$N; Long. 03° 58$^1$E) in tropical rainforest agro-ecological belt. Meanwhile, the first Brazilian cashew population was introduced in 1986 and planted into 350 hectares of land at Kosoni-Ola Farms Limited, Oro, Kwara State (Lat. 08° 26$^1$N; Long. 04° 29$^1$E), in the southern guinea savannah agroecology of Nigeria. Each population was represented by ten accessions.

Root tips for mitotic studies were obtained from nuts of the selected accessions germinated in a rooting medium of topsoil and sawdust mixed in equal proportion. Harvested root tips were pretreated with saturated solution of 8-hydroxyl-quinonine and kept in a dark cupboard at room temperature for 24 h (Morakinyo and Olorode, 1984; Adebola and Morakinyo, 2005). Treated root tips were fixed in aceto-ethanol (1:3 v/v) for 24 h and refrigerated in 70% ethyl-acohol until use. Root tips for observation were hydrolyzed in 1N Hydrochloric acid warmed at 45°C for 5 - 7 min and squashed in 2.0% aceto-carmine stain (Morakinyo and Olorode, 1984).

Flower buds for meiotic studies were collected between 8.00am and 12.00noon, fixed in Carnoy's fluid (ethanol-chloroform-acetic acid, 6:3:1 v/v) for 24 h and squashed in 2.0% aceto-carmine stain (Agarwal and Gupta, 1983; Morakinyo and Olorode, 1984). Alternate heating and cooling of the slide after squashing was done by passing it over the flame of a spirit burner to ensure good spread and well stained chromosome (Barone and Saccardo, 1990).

Preparations were observed with the aid of a Zeiss binocular microscope attached with photographic facilities. Chromosome number was counted at metaphase and pro-metaphase stages from suitable preparations and photomicrographs were taken. Chromosome behaviour in terms of pairing, division during anaphase and the structure were also recorded. Length of the chromosome was measured with aid of a calibrated eyepiece graticule and the karyotype was determined according to Levan et al. (1964), Oyewole (1972) and Agarwal and Gupta (1983).

## RESULTS

The mitotic chromosomes of the studied accessions are presented in Figures 1, 2, 3 and 4 with their corresponding schematic drawings. Diploid chromosome number of 2n = 42 was recorded among the two selected cashew populations. The karyotypic observations on the chromosomes recorded among the Indian and Brazilian populations comprising mostly of medium and large-jumbo sized nuts are summarized in Tables 1 and 2 respectively. The total length of the homologous chromosomes recorded for the Indian cashew population was found to be 51.10 µm, and were designated 1 - 21, according to decreasing lengths. Karyotyping was based on the absolute length of the chromosome and ratio of chromosome short arms to long arms (centromeric location) as suggested by Levan et al. (1964), Oyewole(1972) and Agarwal and Gupta (1983). The chromosome compliment gave a karyotypic formulae of 6Asm + 1Am + 4Bsm + 5Bm + 5Cm, while A represent chromosome $\geq$3.00 µm, B = 1.50 - 2.99 µm and C $\leq$ 1.49 µm (Agarwal and Gupta, 1983). Meanwhile, the chromo-

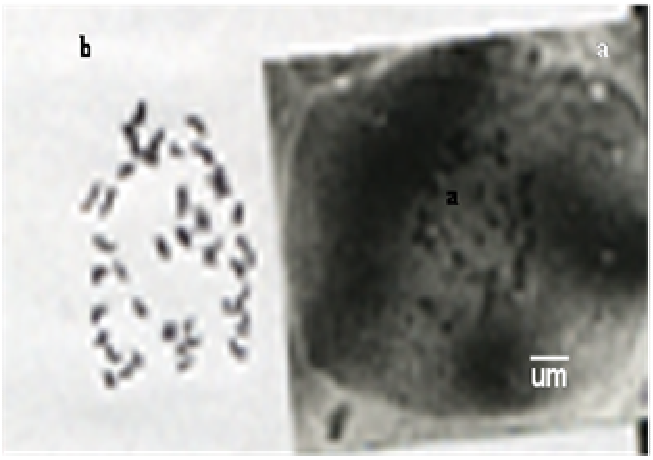



**Figure 1** (a): Mitotic chromosomes of Indian cashew accession. (b): The corresponding schematic drawings. Magnification: x400.

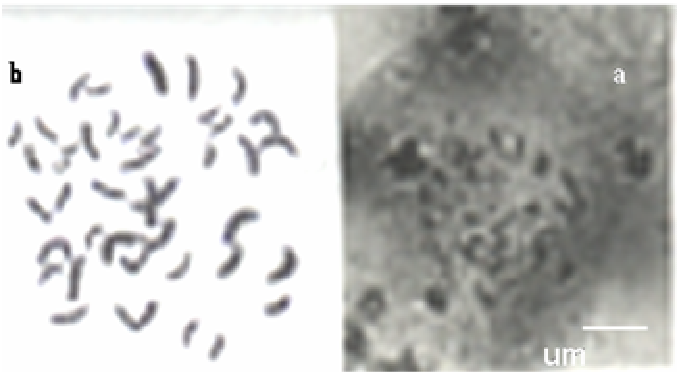



**Figure 3**. (a): Mitotic metaphase chromosomes of Brazilian cashew accession. (b): The corresponding schematic drawings. Magnification: x400.

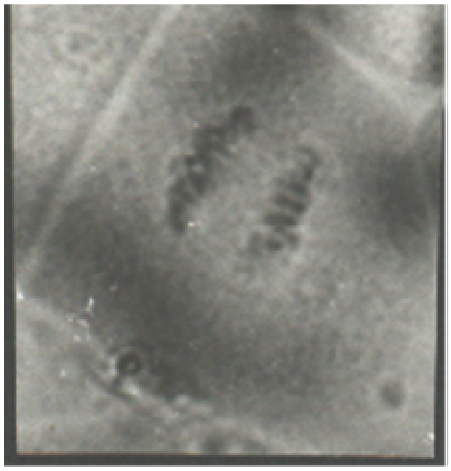


**Figure 2**. Late anaphase of Indian cashew accession showing regular mitotic chromosome division. Magnification: x400.

**Figure 4**. Late anaphase of Brazilian cashew accession showing regular mitotic chromosome division. Magnification: x400.

some lengths ranged between 1.00 and 4.200 µm for the shortest and the longest respectively. Based on the morphology of the chromosomes, the complement comprises of 6 long sub-metacentric, 1 long metacentric, 4 intermediate sub-metacentric, 5 intermediate metacentric and 5 small metacentric chromosomes (Table 1) with regular mitotic division (Figure 2).

The mitotic metaphase chromosome of Brazilian cashew population is presented in Table 2 and Figure 3, with total chromosome length of 56.00 µm. Individual chromosome length ranged between 1.00 and 4.50 µm. The chromosome karyotype was very similar to that of Indian population comprising, 6Asm + 1Am + 1Ast + 9Bm+ 2Bsm + 2Cm. It however shows that the complement includes, 6 long sub-metacentric, 1 long metacentric, 1 long sub-telocentric 9 intermediate metacentric, 2 intermediate sub-metacentric and 2 small metacentric chromosomes with regular mitotic division (Figure 4). Apart from the slight variation in the chromosome length observed between the two populations, the chromosome stainability and behaviour during different stages of cell division was quite similar and regular divisions were recorded in their anaphase phases (Figures 3 and 4).

Regular meiotic divisions of 21 bivalents (Figure 5) were commonly recorded among the two studied population with common occurrence tetrads in their pollen mother cells (Figure 6). This observation is an indication of potential high fertility of the pollen grain of cashew acession in these two populations. Few triads signifying iregularities during meiosis (microsporoge-nesis) was obtained in the pollen mother cells of three Brazilian accessions (Figure 7). The occurrence of triads in the pollen mother cells of these three accessions would probably have implication on the fertility of their pollens.

**Table 1.** Chromosome homologues of Indian cashew accessions.

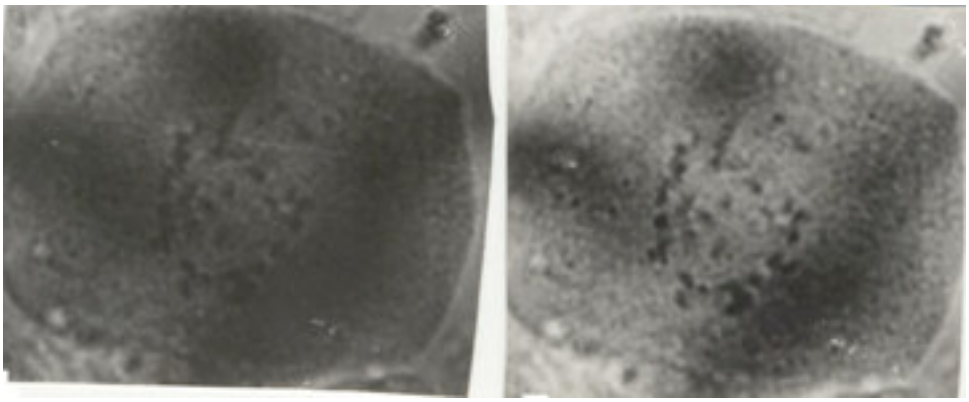
| Chromosome number | Total length (µm) | Long arm (µm) | Short arm (µm) | Arm ratio | Centromere position |
|---|---|---|---|---|---|
| 1 | 4.2 | 3.0 | 1.2 | 2.5 | SM |
| 2 | 4.0 | 3.0 | 1.0 | 3.0 | SM |
| 3 | 3.9 | 2.7 | 1.2 | 2.3 | SM |
| 4 | 3.5 | 2.5 | 1.0 | 2.5 | SM |
| 5 | 3.0 | 2.0 | 1.0 | 2.0 | SM |
| 6 | 3.0 | 2.0 | 1.0 | 2.0 | SM |
| 7 | 3.0 | 1.8 | 1.2 | 1.5 | M |
| 8 | 2.5 | 1.8 | 0.7 | 2.6 | SM |
| 9 | 2.5 | 1.8 | 0.7 | 2.6 | SM |
| 10 | 2.5 | 1.5 | 1.0 | 1.5 | M |
| 11 | 2.5 | 1.5 | 1.0 | 1.5 | M |
| 12 | 2.0 | 1.5 | 0.5 | 3.0 | SM |
| 13 | 2.0 | 1.5 | 0.5 | 3.0 | SM |
| 14 | 2.0 | 1.2 | 0.8 | 1.5 | M |
| 15 | 2.0 | 1.2 | 0.8 | 1.5 | M |
| 16 | 2.0 | 1.0 | 1.0 | 1.0 | M |
| 17 | 1.5 | 1.0 | 0.5 | 2.0 | M |
| 18 | 1.5 | 0.8 | 0.7 | 1.1 | M |
| 19 | 1.5 | 0.8 | 0.7 | 1.1 | M |
| 20 | 1.0 | 0.6 | 0.4 | 1.5 | M |
| 21 | 1.0 | 0.5 | 0.5 | 1.0 | M |

Total length of chromosome complement = 51.10 µm, M: Metacentric; SM: Submetacentric;

**Table 2**. Chromosome homologues of Brazilian cashew accessions.

| Chromosome number | Total length (µm) | Long arm (µm) | Short arm (µm) | Arm ratio | Centromere position |
|---|---|---|---|---|---|
| 1 | 4.5 | 3.2 | 1.3 | 2.5 | SM |
| 2 | 4.3 | 3.0 | 1.3 | 2.3 | SM |
| 3 | 4.0 | 3.0 | 1.0 | 3.0 | SM |
| 4 | 3.8 | 2.8 | 1.0 | 2.8 | SM |
| 5 | 3.8 | 2.5 | 1.3 | 1.9 | SM |
| 6 | 3.2 | 2.5 | 0.7 | 3.6 | ST |
| 7 | 3.2 | 2.0 | 1.2 | 1.7 | M |
| 8 | 3.0 | 2.0 | 1.0 | 2.0 | SM |
| 9 | 2.8 | 1.5 | 1.3 | 1.2 | M |
| 10 | 2.8 | 1.5 | 1.3 | 1.2 | M |
| 11 | 2.8 | 1.5 | 1.3 | 1.2 | M |
| 12 | 2.5 | 1.5 | 1.0 | 1.5 | M |
| 13 | 2.5 | 1.5 | 1.0 | 1.5 | M |
| 14 | 2.0 | 1.5 | 0.5 | 3.0 | SM |
| 15 | 2.0 | 1.2 | 0.8 | 1.5 | M |
| 16 | 2.0 | 1.0 | 1.0 | 1.0 | M |
| 17 | 1.8 | 1.0 | 0.5 | 1.3 | M |
| 18 | 1.5 | 1.0 | 0.7 | 2.0 | SM |
| 19 | 1.5 | 0.8 | 0.7 | 1.1 | M |
| 20 | 1.0 | 0.5 | 0.5 | 1.0 | M |
| 21 | 1.0 | 0.5 | 0.5 | 1.0 | M |

Total length of chromosome complement = 56.00 µm, M: Metacentric; SM: Submetacentric; ST: Submetacentric.



a b

**Figure 5.** Mieotic cell division in Indian (a) and Brazilian (b) cashew accessions showing 21 bivalents.

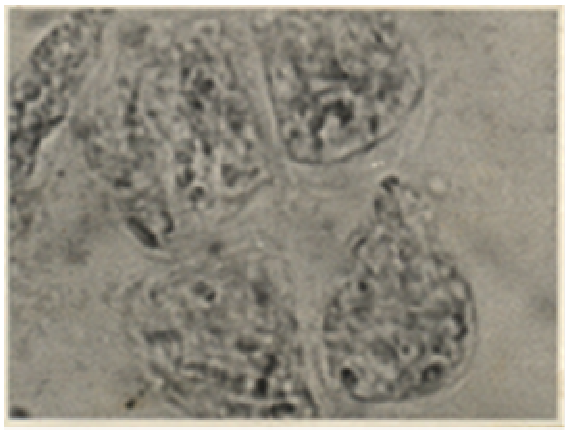


**Figure 6**. Tetrads in pollen mother cell showing regular meiotic division. Magnification: x400.

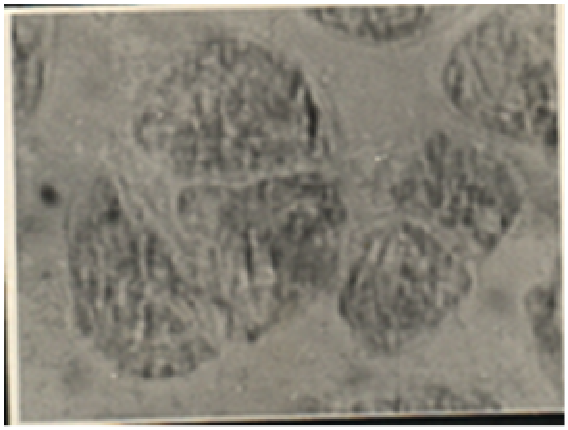


**Figure 7.** Triads showing irregularities in microsporogenesis in Brazilian accessions. Magnification: x400.

## DISCUSSION

The diploid chromosome number of 2n = 42 and haploid of n = 21 obtained in this study agrees with Hutchinson and Dalziel (1954), but disagree with the polymorphism of 2n = 24, 30, 40, and 42 reported by Deckers et al. (2001). Archack et al. (2003) quoted Purseglove (1968) that cashew has a chromosome number of 2n = 42; however, the ploidy is unclear. The accessions are probably polyploids with basic chromosome number x = 7. The chromosome lengths recorded in haploid set in this study tends to group into 3 sets (A B and C of karyotypic formulae), probably corroborating the 7x basic chromosome number. Mabberley (1997) reported basic chromosome number of between 7 and 16 in *Anacardiaceae.* The relative similarity to previous findings probably suggests that the crop species is relatively stable, with very little changes in the chromosomes. The similarity in chromosome number of the accessions in the two evaluated cashew populations also suggest that the materials are morphologically and genomically close, and probably have a common progenitor. The chromosome morphology of these two populations was very similar with respect to the range and gradation of chromosome length and the position of centromere. The chromosomes are mostly metacentric and submetacentric with regular mitotic cell division. Although the chromosomes are relatively small, slight differences in their size and morphology were detected. Most of the accessions analysed for the two populations have symmetric karyotypes, composed mainly of metacentric pairs with several sub-metacentrics. The presence of a pair of sub-telocentric chromosome in the Brazilian popu-lation would seem to be exceptional in the cashew cultivars. This fact probably suggests intra-species varia-tion, accompanied by few changes in the karyotype con-stitiution of the species (Dematteis, 1998). Similar obser-vation was reported on *Cola* species (Morakinyo, 1995). Although the chromosome number in all the accessions

was the same, the total chromatin length varied slightly. Guerra (2000) remarked that heterochromatin content is not homogenous, varying qualitatively and quantitatively between species and within a single
crop species, and polymorphism for the number and size of the bands is frequent. The amount of heterochromatin varies regardless the amount of euchromatin or the nuclear DNA content, and both heterochromatin and euchromatin can suffer large changes in relatively short time. The same crop species may present simultaneously differences in chromosome number, size and morphology, as well as in amount, composition and distribution of heterochromatin (Greilhuber, 1982; Vosa, 1985; John, 1988; Summer, 1990). Morakinyo and Adebola (1991) reported similar results on *Pennisetum purpureum* (green) and *P. purpurem* (purple) with total chromatin length of 107.3 µm and 106.9 µm respectively. Slight variation in chromosome size and total chromatin content was explained using the gene duplication and multiple strand hypotheses by Stebbins (1971). Degree of genomic closeness observed suggests that gene exchange among the accessions could be possible. Regularity of the chromosome behaviour during meiotic division and free flow of gene exchange observed in the fruit set study attest to the commonness of the progenitor of these accessions. Close similarity in the chromosome number, behaviour and structure of these accessions corroborate high degree of similarity in the tree morphological appearance (Aliyu, 2004). Regularity of chromosome behaviour during meiotic cell division probably attests to the high pollen grain fertility recorded among the selections (Aliyu, 2004).

## ACKNOWLEDGEMENT

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